THE ROLE OF BIOFILMS AND CURLI IN ENVIRONMENTAL SALMONELLA TRANSPORT THROUGH POROUS MEDIA

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Anthony Edward Salvucci

January 2009

© 2009 Anthony Edward Salvucci

ABSTRACT

Microbial pathogens, such as *Salmonella* and *Escherichia coli*, are continually deposited in the environment and have been shown to contaminate groundwater by leaching through the vadose zone. Therefore, understanding the factors influencing the transport of these pathogens through porous media is critical to protecting drinking water supplies. This study investigated the relationship between biofilm formation and curli production and the transport of environmental *Salmonella* through porous media. Environmental *Salmonella* strains used in the experiment were isolated from dairy farms. Two well-characterized *E. coli* strains with known high and low biofilm and curli production capabilities were also tested as controls alongside the *Salmonella* isolates for each experiment. Thirty-two isolates were initially assayed for their ability to form biofilms, from which a subset of these was selected to represent a range of high and low biofilm and curli formation capabilities. These were subsequently examined in unsaturated sand columns for transport characteristics. Unlike the *E. coli* strains for which column retention correlated with biofilm formation and curli production, no obvious correlation was observed between *Salmonella* phenotypes and column retention. *Salmonella* strains that produced high amounts of curli and biofilms were actually less retained in the column than *Salmonella* strains producing low amounts of curli and biofilms, contradictory to observations made with *E. coli*. Swimming and swarming motility exhibited by strains also proved to have no correlation with column transport. The results indicate that while transport of well-characterized laboratory *E. coli* strains can often be hindered by the presence of curli and biofilms, the presence of curli did not retard the transport of the *Salmonella* strains through sand columns though it correlated with their ability to form biofilms.

BIOGRAPHICAL SKETCH

Anthony Edward Salvucci was born to Michael and Cynthia Salvucci in Urbana, Illinois in 1983. After growing up in Lexington, Kentucky and Gilbert, Arizona, he attended the University of Arizona, graduating Cum Laude with a Bachelors of Science in Environmental Science in 2006. As a Masters student at Cornell University, he focused on the transport of contaminants in the subsurface, examining the transport of pesticides to the groundwater level in New York and factors influencing bacterial transport through porous media. His other interests are riding bicycles, vinyl records, cassette tapes, ultimate frisbee, camping and rope swings.

This thesis is dedicated to my parents, Michael and Cynthia, and my brother Matthew, for all the love and support they have provided me throughout my life.

ACKNOWLEDGMENTS

I would like to acknowledge my advisors, Dr. Tammo Steenhuis and Dr. Anthony Hay, who provided me with invaluable feedback and guidance throughout the process. I would also like to acknowledge my classmates of BEE 672, Daniel Fuka, Rebecca Majerison, Tong Zhang, Luis Caballero, Wei Zhang, Cody Charwood, Helen Dahlke and Rachael Dunn, who helped initiate and brainstorm this project with me:. Finally, I would like to acknowledge all my colleagues, Brian Campbell, Dr. F. Andreas Toba, Dr. Brian Richards, Wei Zhang, Helen Dahlke, M. Ekrem Cakmak, and Veronica Morales, who assisted me in experimental guidance, editing and revision process.

TABLE OF CONTENTS

Contents **Page**

Contents **Page**

Appendix

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

E. coli	*Escherichia coli*
DLVO	Derjaguin-Landau-Verwey-Overbeek
tafi	thin aggregative fimbriae
LPS	lipopolysaccharides
EDTA	ethylenediaminetetraacetic acid
NaCl	Sodium chloride
LB	Luria-Bertani
M9	M9 salts minimal growth media
OD	Optical Density
ABS	Absorbance
CR	Congo Red
YESCA	yeast extract casamino acids
PBS	phosphate buffered saline
BTC	breakthrough curve
KBr	Potassium Bromide
rdar	red, dry and rough
saw	smooth and white

LIST OF SYMBOLS

µL	microliter
g	grams
L	liter
nm	nanometer
%	percent
ºC	degrees celsius
µm	micrometer
cm	centimeter
mM	micromolar
C’	normalized concentration
C_o	initial concentration
C_f	concentration measured at final time-step
V	effluent volume (µL)
V_f	total volume (µL)
k	each individual sample
n	total number of samples
ppm	parts per million
f	fraction

CHAPTER 1

INTRODUCTION

1.1 Thesis Outline

Chapter 1 provides background information on the fate and movement of bacteria in the subsurface. The second section defines biofilms and curli and provides background on the study of their impact on bacterial transport through the subsurface.

Chapter 2 presents the complete investigation of the impact of biofilms and curli on the transport of *Salmonella* through porous media.

Chapter 3 recommends follow-up investigations and future directions to take regarding the study of subsurface transport of bacteria.

Appendix A contains the actual data from the experimental work examining the transport of bacterial strains in sand columns.

Appendix B has the mass recovery calculations along with their statistical comparisons.

Appendix C contains a complete collection of the statistical summaries.

1.2 Background on bacteria in the subsurface

As far back as the 19th century, microbial tracer populations were introduced and recollected from the subsurface, as a method of tracking groundwater flow patterns (Abba et al. 1898). However, in the past few decades, research has shifted to focus more on the movement of the microbes themselves, as they have implications in bioremediation (Steffan et al. 1999; Ellis et al. 2000) and groundwater contamination (Crane et al. 1983, Ferguson et al. 2003). The protection of groundwater resources is of particular interest, as several recent disease outbreaks (O'Conner 2001; CDC 2008; CDPHE 2008) have been linked to pathogenic bacterial contamination of subsurface drinking water sources. Controlled

experiments (Jackson et al. 1998; Warnemuende & Kandar 2000; Ogden et al. 2001; Juhna et al. 2007) also verify that pathogenic bacteria have the potential to reach and contaminate groundwater, representing a serious human health concern.

Enterobacterial pathogens, such as *Escherichia coli* and *Salmonella*, are continually deposited in the environment from a variety of human and animal sources (Winfield & Groisman 2003). The infiltration of precipitation facilitates the transport of these pathogens to the groundwater level. However, bacterial transport to groundwater is partially impeded by the soil matrix, which acts as a natural microbial filter.

Bacterial subsurface transport is governed by physical, chemical and/or biological processes (Ginn et al. 2002, Yang et al. 2004). Physical processes, like straining and filtration physically retain microbes from the flowing solution. Straining specifically refers to the immobilization of microbes too large to fit through pore space openings (Choi et al. 2007**).** Physical filtration is the collision and subsequent deposition of microbes onto particle surfaces (Murphy & Ginn 2000). These physical processes can also be coupled with chemical interactions between the bacterial cell surface and the mineral surface, resulting in attachment, adsorption, or even repulsion (Ginn et al. 2002). These chemical interactions are highly influenced by the cell surface properties, which can vary significantly among microbial strains (Choi et al. 2007). Active adhesion and detachment are biomolecule-mediated microbial interactions with mineral surfaces and vary in response to nutrient availability, environmental stress, and growth (Bouwer & Cobb 1987; Reynolds et al. 1989), which can influence microbial transport (Ginn et al. 2002).

The physical, chemical and biological processes influencing microbial subsurface transport are inherently complex, due to their unstable and interrelated nature (Ginn et al. 2002). There is not a clear distinction between physico-chemical

and biological mechanisms because these processes are often coupled in relation to microbial transport. Consequently, a definitive and encompassing set of rules and laws governing microbial transport does not exist, as can be seen by the variances and inconsistencies within the literature (Murphy and Ginn 2000; Tufenkji 2007).

Past attempts to predict bacterial transport have traditionally relied upon inorganic colloid theories to explain bacterial interactions with surfaces (Ginn et al. 2002; Tufenkji 2007). Traditional colloid filtration theory describes the transport of the particle through the porous media with the pore-water, whereupon it can attach to the surface of the collector media (Ginn et al. 2002). The interactions that determine whether the particle will attach to the surface are commonly described by the DLVO theory of colloid stability, which balances the attractive and repulsive physiochemical interaction (Grasso et al. 2002). However, many of the DLVO theory's assumptions, in particular that the colloidal surface is smooth, solid, and inert, do not hold true for microbial cells (Ninham 1999) due the presence of cell surface polymers, macromolecules, and other structural appendages that may contribute to biofilm formation.

1.3 Biofilms, Curli, and Bacterial Transport

Upon entry into non-host environments, bacteria are subject to limited nutrient availability, osmostic stress, variations in temperature and pH, and predation (Marshal 1980; Savageau 1983; Winfield & Groisman 2003). In order to enhance survival, cells will often form biofilms (Costerton et al. 1995). Biofilms are organized communities of cells enclosed in an extracellular matrix usually consisting of polysaccharides, proteins and extracellular surface structures such as fimbriae or pili (Costerton et al. 1995). Cells in biofims are typically more resistant to biocides, antibiotics, desiccation and oxidative stress (Hoyle & Costerton 1991, Stewart 2001, Brombacher et al. 2003).

The formation of a biofilm is a multi-step processes. The first step is reversible cell attachment to a surface, followed by irreversible attachment and the production of adhesive molecules (Barnhart & Chapman 2006). After attachment, the biofilm begins to develop, then matures, before finally dispersing (Barnhart and Chapman 2006). This process of biofilm formation is assisted by the presence of several bacterial surface structures, such as curli, flagella, pili, and exopolysaccharides (Van Houdt & Michiels 2005) as they facilitate the initial attachment to surfaces (Deflaun et al. 1994; Vidal et al. 1998).

One of the key surface structures are some enteric bacteria are curli. Curli are proteinaceous extracellular fibers produced by enteric bacteria (Barnhart and Chapman 2006). When found in *Salmonella*, these amyloid fibers are often referred to as thin aggregative fimbraie (tafi) (Gebbink et al. 2005). For the sake of clarity, similar amyloid fibers expressed by both *E. coli* and *Salmonella* will be referred to as curli throughout this thesis. The expression of curli promotes both surface and cell-cell adhesion, which can lead to biofilm formation and host colonization (Prigent-Combaret et al. 2001; Zogaj et al. 2003). Curli gene expression is generally responsive to environmental cues such as temperature and salt concentrations (Römling et al. 1998b), and is highly conserved between *E. coli* and *Salmonella* (Römling et al. 1998a).

The production of extracellular structures, like curli, facilitates bacterial attachment to surfaces (Marshall 1980; Lynch & Bragg 1985). Upon surface attachment, the process of biofilm formation can begin (Barnhart & Chapman 2006). As curli also increases cell-to-cell attachment they contribute to biofilm development beyond the attachment stage. It is logical to hypothesize that the presence of curli and its associated biofilm formation phenotype would retard the transport of bacteria through the soil, as the cell becomes attached to the grains. This hypothesis is largely supported by findings in the literature (Prigent-Combaret

et al. 2001; Landini & Zehnder 2002; Abu-Lail & Camesano 2003; Brombacher et al. 2003; Burks et al. 2003; Walker et al. 2004; Bell et al. 2005). Both the physical removal of LPS (Abu-Lail & Camesano 2003) and gene deletion to inactivate curli production (Brombacher et al. 2003) were shown to lead to increase transport of *E. coli* through saturated columns. However, other factors such as variations in strain type, culture age and media condition can often overcome and/or alter the influence of surface biomolecules and biofilm formation capacity on the transport of bacterial through the porous media (Rijnaarts et al. 1995; Walker et al. 2005; Tufenkji 2007). For example, Rijnaarts et al. (1995) showed ionic strength to be a key factor in bacterial retention, while Walker et al. (2005) found that differences in the growth phase of the cell could overcome differences in ionic strength to influence column retention. In addition, bacterial transport behavior can vary significantly between commonly used laboratory-cultured strains and environmental isolates (Yang et al. 2006).

The exact role of surface biomolecules, like biofilm and curli, in bacteria transport through porous media is still quite poorly understood (Tufenkji 2007). Further complicating this understanding is the fact that the wide variety of bacterial surface polymers can exert either repulsive or attractive forces, depending on their specific characteristics and other environmental factors (Ginn et al. 2002). As a result, well-controlled laboratory-scale studies are needed to improve the fundamental understanding of interactions between surface biomolecules, biofilm formation and porous media. Column studies have been used to examine specific processes that affect microbial transport (Harvey & Harms 2002). Flow-through column investigations mimic subsurface flow and yield value information about the acting retention mechanisms governing bacterial transport, while also providing a degree of control unattainable in the field (Harvey & Harms 2002).

This report attempts to further address the role of surface biomolecules on bacterial transport through porous media using sand columns. We focus specifically on the effects of biofilm formation and curli production on the transport of environmental *Salmonella* strains.

REFERENCES

Abba F., Orlandi, Rondelli A. 1898. Über die Filtrationskraft des Bodens und die Fortschwemmung von Bakterien durch das Grundwasser. Z. Hyg. Infekt. Krankh. **31:** 66–84.

Abu-Lail N. I., Camesano T. A. 2003. Role of Lipopolysaccharides in the Adhesion, Retention, and Transport of *Escherichia coli* JM109. Environmental Science & Technology **37:** 2173-2183.

Barnhart M. M., Chapman M. R. 2006. Curli Biogenesis and Function. Annual Review of Microbiology **60:** 131-47

Barton J. W., Ford R. M. 1995. Determination of effective transport coefficients for bacterial migration in sand columns. Applied Environmental Microbiology **61:** 3329-3335.

Bell C. H., Bhupinder S. A., Camesano T. A. 2005. Adhesion of *Pseudomonas putida* KT2442 is mediated by surface polymers at the nano- and microscale. Environmental Engineering Science. **22:** 629-641

Bouwer E. J., Cobb G. D. 1987. Modeling of biological processes in the subsurface. Water Science &Technology **19:** 769 – 780.

Brombacher E., Dorel C., Zehnder A. J., Landini. P. 2003. The curli biosynthesis regulator CsgD co-ordinates the expression of both positive and negative determinants for biofilm formation in *Escherichia coli.* Microbiology **149:** 2847–2857.

Burks G. A., Velegol S. B., Paramonova E., Lindenmuth B. E., Feick J. D, Logan B. E. 2003. Macroscopic and nanoscale measurements of the adhesion of bacteria with varying outer layer surface composition. Langmuir **19:** 2366–2371.

Centers for Disease Control and Prevention (CDC). 12 Aug. 2008. Investigation of outbreak of infections caused by *Salmonella* Saintpaul. <http://www.cdc.gov/salmonella/saintpaul/>

Choi N.-C., Kim D.-J., Kim S.-B. 2007. Quantification of bacterial mass recovery as a function of pore-water velocity and ionic strength. Research in Microbiology **158:** 70-78.

Colorado Department of Public Health and Environment (CDPHE). 21 Mar. 2008. New Release "Officials provide update on bottled water order" <http://www.cdphe.state.co.us/release/2008/03212008.html>

Costerton J. W., Lewandowski Z., Caldwell D. E., Korber D. R., Lappin-Scott H. M. 1995. Microbial biofilms. Annual Review of Microbiology **49:** 711–745.

Crane S. R., Moore J. A., Grismer M. E., Miner J. R. 1983. Bacterial pollution from agricultural sources: A review. Transactions of the ASAE **26:** 858-866.

DeFlaun, M. F., B. M. Marshall, E.-P. Kulle, and S. B. Levy. 1994. Tn5 insertion mutants of *Pseudomonas fluorescens* defective in adhesion to soil and seeds. Applied Environmental Microbiology 6**0:** 2637–2642.

Ellis D. E., Lutz E. J., Odom J. M., Buchanan Jr R. J., Bartlett CL, Lee M. D., et al. 2000. Bioaugmentation for accelerated in situ anaerobic bioremediation. Environmental Science &Technology. **34:** 2254-2260.

Ferguson C., de Roda Husman A. M., Altavilla N., Deere D., Ashbolt N. 2003. Fate and transport of surface water pathogens in watersheds. Critical Reviews in Environmental Science & Technology **33:** 299–361.

Gibbink M. F. B. G., Claessen D., Bouma B,. Dijkhuizen L., Wosten H. A. B. 2005. Amyloids—a functional coat for microorganisms. Nature Reviews Microbiology **3:** 333−341

Ginn T.R., Wood B. D., Nelson K. E., Scheibe T. D., Murphy E. M., Prabhakar Clement T. 2002. Processes in microbial transport in the natural subsurface. Advances in Water Resources 2**5:** 1017-1042.

Grasso D., Subramaniam K., Butkus M., Strevett K., Bergendahl L. 2002. A review of non-DLVO interaqctions in environmental colloidal systems. Environmental Science and Technology **1:** 17-38.

Harvey R. W., Harms H. 2002 Transport of microorganisms in the terrestrial subsurface: in situ and laboratory methods. In: Hurst CJ, editor. Manual of environmental microbiology. Washington, DC: ASM Press; p. 1138.

Hoyle, B. D., Costerton, W. J. 1991. Bacterial resistance to antibiotics: the role of biofilms. Progress in Drug Research **37:** 91–105.

Jackson, S. G., Goodbrand, R. B., Johnson, R. P., Odorico, V. G., Alves, D., Rahn, K., et al. 1998. *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. Epidemiology & Infection **120:** 17–20.

Juhna T., Birzniece D., Larsson S., Zulenkovs D., Sharipo A., Azevedo N.F. et. al. 2007. Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks. Applied Environmental Microbiology. **73:** 7456-7464.

Landini P., Zehnder A. J. 2002. The global regulatory hns gene negatively affects adhesion to solid surfaces by anaerobically grown *Escherichia coli* by modulating the expression of lipopolysaccharide and flagellar genes. Journal of Bacteriology **184:** 1522-1529.

Lynch J. M., Bragg E. 1985. Microorganisms and soil aggregate stability. Advances in Soil Sciences **2:** 133–171.

Marshall, K. C. 1980. Adsorption of microorganisms to soils and sediments, p. 317–329. In G. Britton and K. C. Marshall (ed.), Adsorption of microorganisms to surfaces. Wiley, New York, N.Y.

Murphy E. M., Ginn T. R. 2000. Modeling microbial processes in porous media. Hydrogeology Journal **8:** 142-158

Ninham B. W. 1999. On progress in forces since the DLVO theory. Advances in Colloid & Interface Science **83:** 1–17.

O'Connor R. 2001. Report of the Walkerton Inquiry. Toronto, ON: Publications Ontario. Bioresource Technology **54:** 165-169.

Ogden I. D., Fenlon D. R., Vinten A. J. A., Lewis D. 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. International Journal of Food Microbiology **66:** 111-117

Prigent-Combaret C., Brombacher E., Vidal O., Lejeune P., Ambert A., Landini P., Dorel C. 2001. A complex regulatory network controls initial adhesion and biofilm formation in Escherichia coli via regulation of the csgB gene. Journal of Bacteriology **183:** 7213–7223.

Reynolds P. J., Sharma P., Jenneman G. E., McInerney M. J. 1989. Mechanisms of microbial movement in subsurface materials. Applied Environmental Microbiology **55:** 2280–2286.

Römling U., Bian Z., Hammar M., Sierralta W., Normark S. 1998a. Curli fibers are highly conserved between *Salmonella typhimurium* and *Escherichia coli* with respect to operon structure and regulation. Journal of. Bacteriology **180:** 722–731

Römling U., Sierralta W. D., Eriksson K., Normark S. 1998b. Multicellular and aggregative behavior of *Salmonella typhimurium* strains is controlled by mutations in the agfD promoter. Molecular Microbiology. **28:** 249–264 .

Savageau, M. A. 1983. *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. American Naturalist **122:** 732–744.

Steffan R. J., Sperry K. L., Walsh M. T., Vainberg S., Condee C. W., 1999. Field-scale evaluation of in situ bioaugmentation for remediation of chlorinated solvents in groundwater. Environmental Science & Technology **33:** 2771-2781.

Stewart P. S. 2001. Multicellular resistance: biofilms. Trends in Microbiology **9:** 34–39.

Tufenkji N. 2007. Modeling microbial transport in prous media: Traditional approaches and recent developments. Advances in Water Resources **30:** 1455-1469.

Van Houdt R., Michiels C. 2005. Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. Research in Microbiology. **156:** 626–633.

Vidal O., Longin R., Prigent-Combaret C., Dorel C., Hooreman M., Lejeune P. 1998. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression. Journal of Bacteriology **180:** 2442–2491

Walker S. L., Redman J. A., Elimelech M. 2004. Role of cell surface lipopolysaccharides (LPS) in *Escherichia coli* K12 adhesion and transport. Langmuir **20:** 7736–7746.

Warnemuende, E. A., Kanwar R. S. 2000. The effect of swine manure application on bacterial quality of leachate from intact soil columns. ASAE Paper No. 00-2053. St. Joseph, MI: ASAE.

Winfield M. D., Groisman E. A. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Applied Environmental Microbiology **69:** 3687–3694.

Yang H. H., Vinopal R. T., Grasso D., Smets B. F., 2004. High diversity among environmental *Escherichia coli* isolates from a bovine feedlot. Applied & Environmental Microbiology **70:** 1528-1536.

Zogaj X., Bokranz W., Nimtz M., Römling U. 2003. Production of cellulose and curli

fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract. Infection & Immunity. **71:** 4151–4158.

CHAPTER 2

THE ROLE OF BIOFILMS AND CURLI IN ENVIRONMENTAL *SALMONELLA* TRANSPORT THROUGH UNSATURATED POROUS MEDIA

2.1 Introduction

Groundwater is considered one of the safest sources of drinking water and is often used without treatment in many households that do not have access to municipal drinking water systems. However, the safety of potable groundwater is under scrutiny, with pathogen contamination of groundwater estimated to cause as many as 5 million illnesses annually in the United States of America alone (Macler & Merkle 2000). In countries where sanitary systems are failing or nonexistent, the number of pathogen infections from groundwater is likely an order of magnitude larger. Microbial contamination of food sources is another pathway of human health infection. In one recent case, a widespread *Salmonella* outbreak infecting more than 1400 individuals in over 43 states (CDC 2008) was linked to contamination of irrigation water sources. Despite our best efforts to prevent water-related diseases, enterobacterial outbreaks occur unabatedly (O'Conner 2001; CDPHE 2008). Our failure to curb such outbreaks is due in part to our lack of understanding exactly how pathogenic bacteria move in the environment (Filip et al. 1988; Stevik et al. 2004; US EPA 2006).

Enterobacteria, including pathogenic *E. coli* and *Salmonella* strains, are commonly found in the intestinal tract and feces of mammals. Fecal deposits of enterobacteria are introduced to the environment through livestock grazing, leaking septic systems, and the spreading of manure and sewage sludge wastes on agricultural fields (Ogden et al. 2001; Jamieson et al. 2002). Upon excretion to the natural environment, pathogenic enterobacteria, such as *E. coli* and *Salmonella*, can

survive for extended periods of times (Gerba et al. 1975; Filip et al. 1998; Entry et al. 2000) thus increasing the likelihood of subsequent infection.

Microbial transport in the subsurface is governed by a multitude of complex and interrelated physical, chemical and biological processes (Ginn et al. 2002). The presence of extracellular structures, such as flagella, pili, curli, and polysaccharides, can have key implications for bacterial transport in the subsurface (Stevik et al. 2004). In addition, these extracellular structures can facilitate the formation of biofilms where bacteria are protected from environmental stresses (Costerton et al. 1995; Van Houdt & Michiels 2005) and which can enhance survival rates in the presence of disinfectants (Prouty et al. 2002; White et al. 2006).

Only a small fraction of research on the effect of biofilms and extracellular structures on bacterial adhesion has been performed using column studies. However, results of these studies are inconclusive, as the presence of extracellular structures has been found to both hinder and facilitate microbial adhesion depending on variations of the structure itself, the strain type, culture age and media condition (Rijnaarts et al. 1995; Abu-Lail & Camesano 2003; Burks et al. 2003; Walker et al. 2005; Tufenkji 2007). Despite this ambiguity, there seems to be an assumption that under specific conditions various biofilm-associated surface structures retard bacterial transport through porous media. For example, *E. coli* strains treated with EDTA to physically remove the membrane-bound lipopolysaccharides (LPS), showed less retention in saturated columns than untreated *E. coli* strains (Abu-Lail & Camesano 2003). Similarly, curli-deficient *E. coli* mutants displayed much higher mobility in sand columns than the wild type did (Brombacher et al. 2003). Similar extracellular-structure effects on transport were found by Prigent-Combaret et al. (2001), Landini & Zehnder (2002), Walker et al. (2004) and Bell et al. (2005).

The most commonly used indicator organism for enterobacterial presence and behavior is *E. coli* due to difficulty and costs involved in testing for a range of

individual pathogens (Crane et al. 1981; Jamieson et al. 2002; Bjergbaek & Roslev 2005). Despite its ubiquitous use, doubts remain about the reliability of *E. coli* as a surrogate for all microbial pathogens, even for similar species like *Salmonella* (Harvey & Helms 2002; Winfield & Groisman 2003). In several instances, *Salmonella* has shown to be more resistant than *E. coli* to environmental stresses, displaying higher rates of survival in natural environments including drinking water sources (Rhodes & Kator 1988; Mezrioui et al. 1995; Winfield & Groisman 2003).

The objective of this study was thus to determine if easily observed interrelated phenotypes of biofilm formation and curli production in *Salmonella* are predictive of transport through sand columns as has been found for *E. coli*. More generally, these experiments were designed to shed light on the extent to which *E. coli* serves as a reliable indicator of the environmental behavior of other enterobacteria and specifically *Salmonella.*

2.2 Material and Methods

2.2.1 Bacterial Selection and Characterization

Salmonella isolates were obtained from the collection of Dr. Lorin Warnick, College of Veterinary Medicine, Cornell University (Kaneene et al. 2008). These Salmonella isolates were originally collected from dairy farms throughout the United States.

Table 1: Bacterial strains used in this study

	Description	**Source/Reference**
E. coli		
PHL 628	MG1655 malA-kan ompR234:Km	Vidal et al. 1998
ΔcsgA	MG1655 malA-kan ompR234 *ΔcsgA*:Cm	Toba et al. 2008
Salmonella		
1-10	unidentified serotype	L. Warnick, Kaneene et al. 2008
1-17	Typhimurium	L. Warnick, Kaneene et al. 2008
1-38	unidentified serotype	L. Warnick, Kaneene et al. 2008
1-46	Schwarzengrund	L. Warnick, Kaneene et al. 2008

The two K-12 *E. coli* strains used in our study were well-characterized laboratory strains, PHL628 and PHL628 Δ*csgA* (Vidal et al.1998; Toba 2008). PHL628 is known to overproduce curli, and Δ*csgA* is a curli deficient deletion mutant form of PHL628 (Toba 2008). All strains were routinely maintained on Luria-Bertani (LB) agar.

Biofilm Formation: The amount of biofilm produced by each strain was measured using a modified version of the method of O'Toole and Kolter (1998). Briefly, in a microtiter plate, 20 μL of an overnight culture of bacteria grown in low-salt (5g/L NaCl) LB broth was added to 100 μL M9 medium (Sambrook et al. 1989) with 0.2% casamino acids as carbon source. After 18 hours incubation at 30° C, bacterial growth was measured by reading the optical density of each well using a 96 plate reader (Biotek) at 600 nm (OD_{600}). Each well was subsequently stained with 100 μL of 1% crystal violet solution. After 15 minutes of staining, the wells were gently washed with DI water until the runoff water was clear, leaving only the stained biofilm residue that was securely attached to the surfaces of the well. After air-drying of the plate, the stain retained by the biofilm growth was solubilized in 95% ethanol and transferred to a fresh plate. The absorbance of each well at the crystal violet absorbance wavelength (595nm) was then measured. The amount of crystal violet retained in each well (ABS_{595}) was normalized to cell growth value (OD_{600}) and presented as the standardized ratio of ABS_{595}/OD_{600}. These assays were performed in triplicate, and the results are presented as averages across all trials.

Congo Red Morphotype Identification: The binding of the dye Congo Red (CR) to *E. coli* and *Salmonella* has been reported to correlate with their production of curli (Römling et al. 1998; Barnhart & Chapman 2006). We therefore plated the four Salmonella strains and the control on Congo Red agar as a qualitative means of assessing curli production. A single colony from LB agar was transferred onto the

center of a YESCA-Congo Red agar plate (Hammar et al. 1996). After 168 hours of incubation at 30º C, colony growth formed by each strain (morphotype) was captured with a digital camera (Canon Powershot A590). Attempts to quantify the amount of Congo Red bound within the morphotype were unsuccessful. To overcome this limitation we performed a semiquantitative visual assessment of congo red absorption by scoring colonies as either a 1 (white), 2 (<25% red), 3 (>25% but <50%), 4(>50% but <80%), or 5 (all red).

Swimming and Swarming Mobility: Swimming and swarming motility plates were made using LB agar, with 0.2% and 0.5% agar concentrations, respectively (Harshay & Matsuyama, 1994). Swarm plates were supplemented with an additional 0.5% glucose concentration after autoclaving. Freshly poured plates were stored in cooler with high air moisture content to reduce desiccation of agar surface. After cooling, 5 µL of an overnight culture of bacteria was inoculated in the center of the agar surface. In swarm plates the bacteria were placed atop the agar surface, while in swim plates, bacteria were injected into the agar using a pipet tip. Plates were sealed with parafilm and placed it a 37° C incubator. The maximum diameter of the spreading growth was measured over various time intervals. Experiments were performed in quadruplicate.

Statistical Analysis of Correlation: Statistical analysis of the correlation between independent biological characteristics was performed with SPSS 16.0 software. Correlation was determined using the Pearson product-moment correlation coefficient, which is obtained by dividing the covariance of the two variables by the product of their standard deviations.

2.2.2 Column Transport Experiments

The four selected *Salmonella* strains and two *E. coli* controls were run through unsaturated sand columns to measure the strains' retention.

Two types of suspension fluid were tested in the sand columns, a growth

media, and a non-growth buffer, in order to observe differences under growth promoting and non-growth environmental conditions. Column effluent breakthrough curves were compared to determine if the ability of a strain to form biofilms and curli was an accurate predictor of the strain's ability to be transported through porous media.

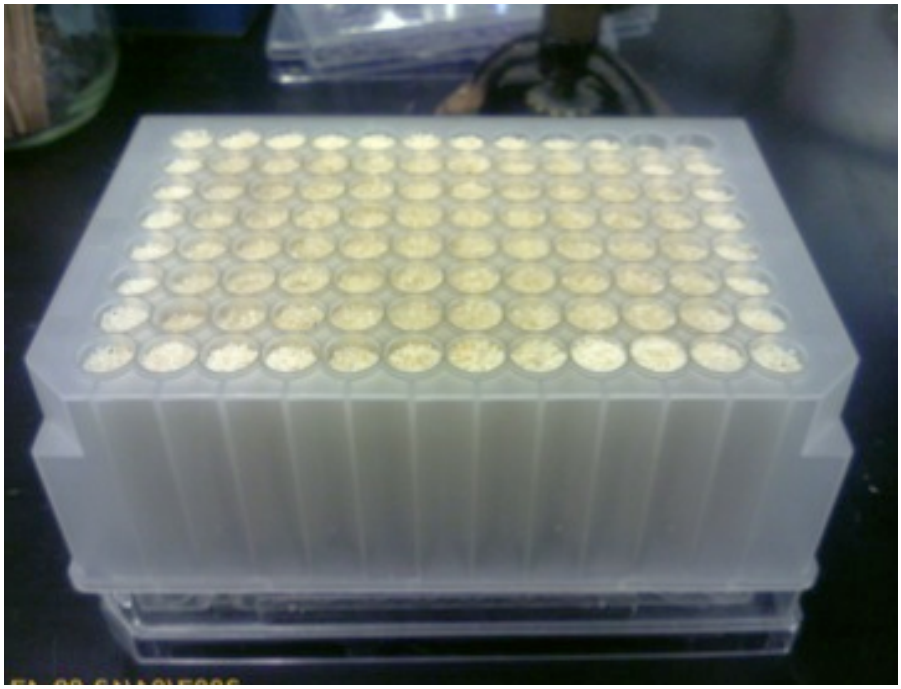


Figure 1: Sand Column set-up, constructed from 96-deep-well plate. Columns are approximately 7 cm in height with a 1 cm circumference and a volume of 2.2 mL.

Sand Column Setup and Construction*:* Granular silica sands with sizes of 500-590 μm and 710-850 μm (Unimin corp.) were used to pack sand columns. Before use, the sand was autoclaved, washed 4 times with DI water and subsequently dried for 24 hours. The sand column unit was constructed from a 96 deep well plate (Axygen Scientific) consisting of 96 individual 2.2mL cylindrical columns. Prior to use, holes of 1mm diameter were drilled at the nadir of the hemispherical bottom of each well. Column set-up can be seen in Figure 1. First,

0.25 g of the coarser sand grain (710-850 μm) was placed in each well in order to serve as a natural filter. Then each column was filled with 2.65 g of the finer sand grains (500-590μm).

Bacterial Preparation: Bacteria were grown in LB broth at 150 rpm at 25º C for 18 hours. Bacterial suspensions were centrifuged to separate whole cells from the LB growth medium. After centrifugation, the medium was decanted and the cells washed by by gently resuspending them in a medium consisting of either a 0.1x (18.6mM) Phosphate Buffered Saline (PBS) or M9 salts medium (0.2% casamino acids as carbon source). This washing procedure was repeated once to remove all traces of the original growth medium and ensure total suspension in the new medium.

PBS Sand Column: 600μL of the PBS inoculated bacterial solution were pulsed into each unsaturated sand column well in eight replicates. In addition, 100μL of the bacterial solution were simultaneously placed in a separate microtiter collector plate, in order to determine the initial cell concentration, measured as optical density (OD_{600}). The column and plate remained sealed and static at room temperature for 4 hours to allow the bacterial suspension to settle in the column. After the 4 hours, the OD_{600} was again measured in the initial OD microtiter plates to verify no growth had occurred in the PBS suspended solutions. Using a multi-channel pipetter, columns were subjected to an initial pulse of 200μL sterile 0.1x PBS, followed by 100μL increments approximately every 3 min. Effluent samples were collected by placing the sand column atop a clean 96-well microtiter plate where approximately 100μL of drained effluent was collected from each well per pulse. The effluent concentrations were analyzed with spectrophotometry and the values normalized and reported as:

$$C' = \frac{C}{C_o} \tag{1}$$

where C' is the normalized concentration, C is the collected column effluent concentration (OD_{600}), and C_o is the initial concentration (OD_{600}) obtained from the separate microtiter plate. The normalized concentrations of each pulse were averaged over all replicates and subsequently plotted against the volume of recovered effluent from each column to generate the breakthrough curve (BTC).

M9 Media Sand Column: These column tests followed the same procedure as the PBS sand columns. The normalized concentration were computed differently in order to account for the small amount of bacterial growth while in column and reported as:

$$C' = \frac{C}{C_f} \tag{2}$$

Where C' is the normalized concentration, C is the collected column effluent concentration (OD_{600}), and C_f is the concentration of the separate microtiter plate (OD_{600}) 4 hours after addition of cells to the column.

Mass Balance: For mass balance calculation, the fraction, *f,* of applied bacteria recovered from the columns was calculated as:

$$f = \frac{\sum_{k=0}^{n} \frac{C'_k + C'_{k+1}}{2}(V_{k+1} - V_k)}{V_t} \tag{3}$$

where C' is the normalized concentration, V is the effluent volume (µL),V_t is the total volume of bacteria pulsed into column, *k* is each sample, and *n* is the total number of samples.

KBr conservative tracer: A Potassium Bromide (KBr) conservative tracer (100 ppm) was run through sand columns under exact same conditions as bacterial strains. Recovered effluent volumes were run through Ion Chromatograph (Dionex) to determine Bromide recovery. Bromide ion recovery was calculated as:

$$C' = \frac{C}{C_o} \tag{4}$$

where C' is the normalized concentration, C is the collected column effluent concentration (ppm), and C_o is the initial concentration (100 ppm).

Statistical Analysis: Using SPSS 16.0 statistical software, statistical analysis was performed on the mass recovery concentrations, using a minimum of 18 column replicates taken from 2 separate column studies. Student t-tests were used to determine significance between strains at the 95% confidence interval. One-sample t-tests assuming unequal variances were performed in which mass recovery was the dependant variable and strain type was the independent variable.

2.3 Results

2.3.1 Biofilm Formation

Thirty-two environmental *Salmonella* strains were analyzed for their biofilm forming abilities (Figure 2). Based on these initial results we selected the two best (*1-38, 1-46*) and two of the worst (*1-10, 1-17*) biofilm formers to examine for additional biofilm related phenotypes, including congo red binding and retention/transport in sand columns.

Figure 3 shows the biofilm produced by the selected subset of *Salmonella sp.* strains along with the *E. coli* controls and composite average of all 32 tested strains. The best *Salmonella* biofilm producing strains, *1-38* and *1-46*, had average measured biofilm absorbance (OD_{595}/OD_{600}) values of 1.69 and 1.50, respectively. Though not statistically different from one another, this was significantly more ($P < 0.05$) than the low biofilm producing strains, *1-10* and *1-17* which had average measured biofilm absorbance (ABS_{595}/OD_{600}) values of 0.37 and 0.35, respectively. Compared to the average of all tested *Salmonella* strains, the high-biofilm producing strains produced more than twice the measured all-strain mean of 0.73, while the low-biofilm producing strains produce approximately half of the mean. Even the best Salmonella biofilm formers, however, produced less biofilm than the curli-overproducing strain (PHL628), which had an ABS_{595}/OD_{600} ratio of approximately

3.83. While the *csgA* deletion mutant produced less biofilm than the wild-type PHL628, it was statistically similar to the biofilm production displayed by the high-biofilm forming *Salmonella* strains (*1-38* and *1-46*) with an ABS_{595}/OD_{600} ratios of approximately 1.6.

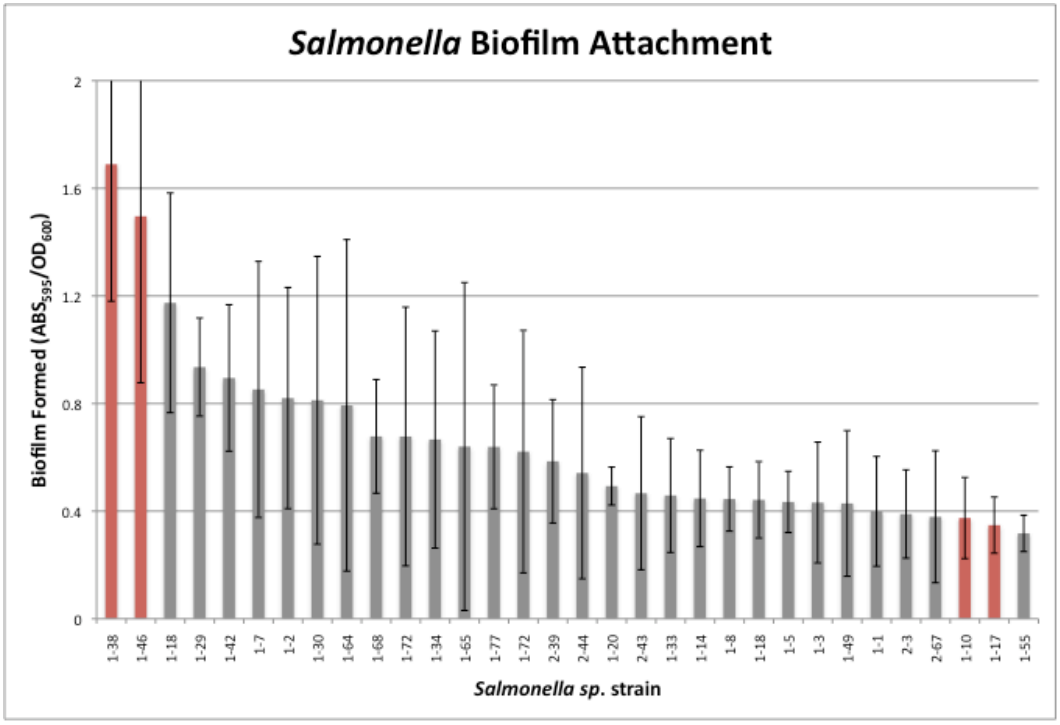


Figure 2: Biofilm attachment summary of all 32 tested *Salmonella* strains measured as the amount of crystal violet stained biofilm attached to polystyrene tray (ABS_{595}) normalized by total cell growth (OD_{600}). Minimum of 3 replicates per strain with error bars representing one standard deviation from the mean.

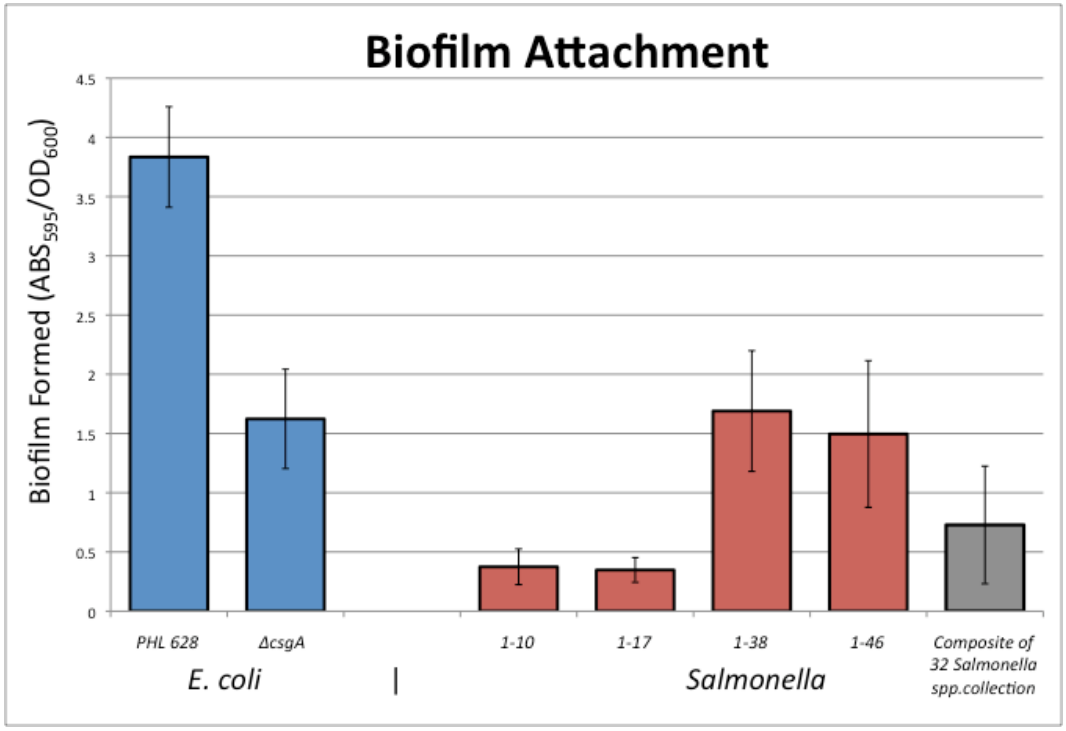


Figure 3: Absorbance of crystal-violet retained by the biofilm attached to polystyrene tray (ABS_{595}), normalized by total cell growth (OD_{600}). *E. coli* strains are blue columns and *Salmonella* strains are red. Composite average of all 32 tested *Salmonella* strains column is shown in gray. Results presented are the mean of 3 independent experiments with a minimum of 3 replicates per experiment (n=9). Error bars represent one standard deviation from the mean.

2.3.2. Congo Red Morphotype Identification

Two distinctly different morphotypes were observed in the colonies formed by the *E. coli* strains: PHL628 produced a red, smooth morphotype (Figure 4a), while *ΔcsgA* produced a smooth and white (*saw*) morphotype (Figure 4b). PHL628 is known to overproduce curli **(**Vidal et al. 1998**)**, and the red morphotype we detected from this strain is consistent with the overproduction of curli. In contrast, the *saw* morphotype formed by *ΔcsgA* was consistent with the absence of curli on the cell surface (with the absence of the *csgA* gene rendering the mutant strain unable to produce curli).

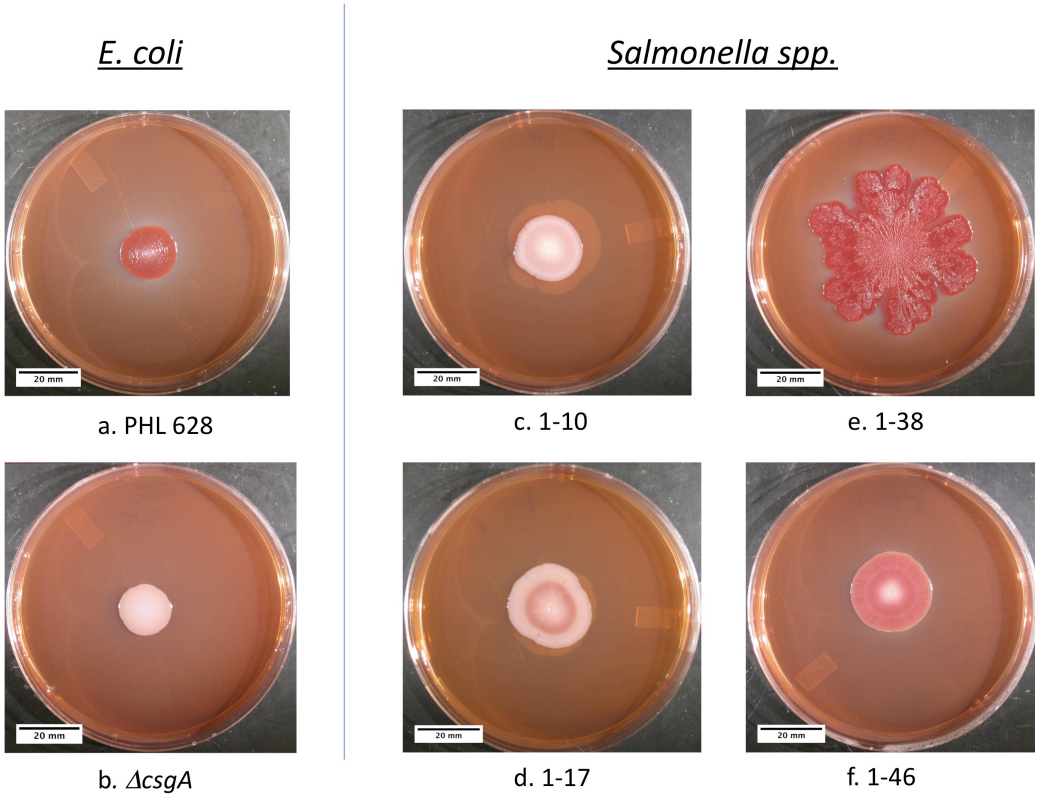


Figure 4: Congo Red Morphotype Images. All colonies were grown at 30°C for 7 days. Images are of *E. coli* strain PHL628 (a) and ΔcsgA (b), *Salmonella* sp. 1-10 (c), 1-17 (d), 1-38 (e) and 1-46 (f).

Upon establishing the two morphotypic extremes displayed by the *E. coli* strains, the environmental isolates of *Salmonella* were analyzed. *Salmonella sp. 1-38* (Figure 4f), produced a red, dry, and rough (*rdar*) morphotype, which indicated the production of both curli and cellulose. The presence of cellulose is known to give rise to the rough ridges (Zogaj et al. 2003) as seen with *1-38*, but not with PHL628 strain which is unable to produce cellulose. The colonies formed by the other three *Salmonella* strains (Figure 4c, 4d, 4f) were not as visually distinct in morphotype as either *Salmonella sp. 1-38* or the *E. coli* strains. Instead of clear *rdar* or *saw* morphotypes, these strains showed intermediate morphotypes, with some red binding indicating the presence of curli but at levels substantially less than PHL628 and *Salmonella sp. 1-38*. The presence of red in these colonies often was higher in some regions of the colony growth, as observed by the ring of red on *1-17* (Fig. 2d)

and the white inner circle on *1-46* (Figure 4f). This unusual distribution of red in only parts of the colony has not been previously reported.

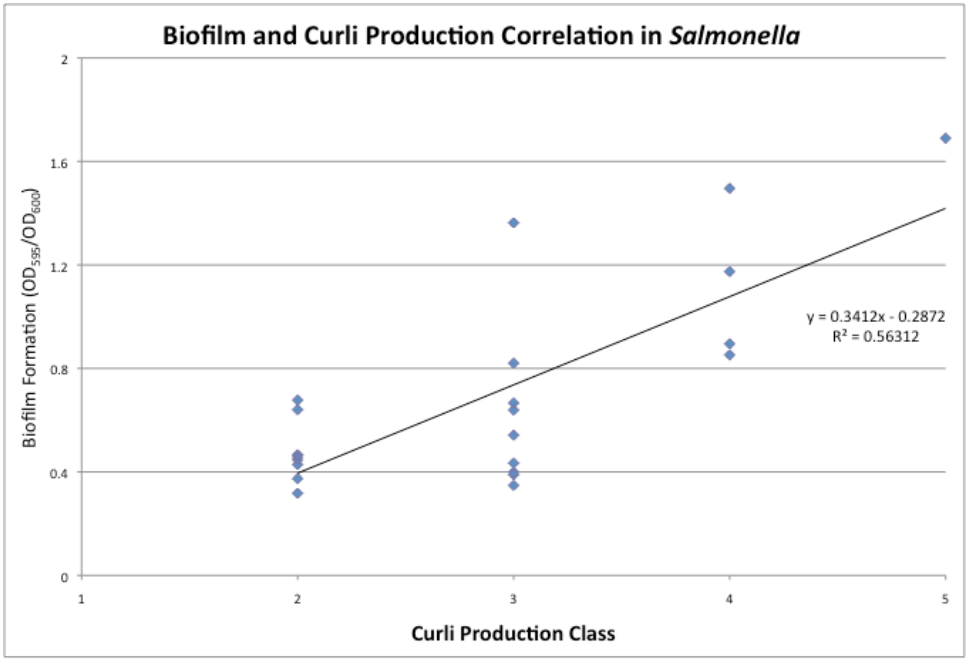


Figure 5: Semiquantitative correlation between biofilm formation and congo red absorption classes in 32 tested *Salmonella* strains. Linear regression line and equation are shown along with R^2. Correlation is statistically significant at the 99% confidence interval.

There was significant correlation ($P < 0.05$) between the observed congo red morphotypes and biofilm formation among the large collection of *Salmonella* strains (Figure 5, Appendix C.4). When examining solely the Salmonella strains tested in the columns, the correlation between biofilm formation and curli production was still significant, verifying that the production of curli is predictive of biofilm formation. However, this correlation was only significant within genera, as the correlation was not significant when the *Salmonella* and *E. coli* strains were grouped (Figure 6).

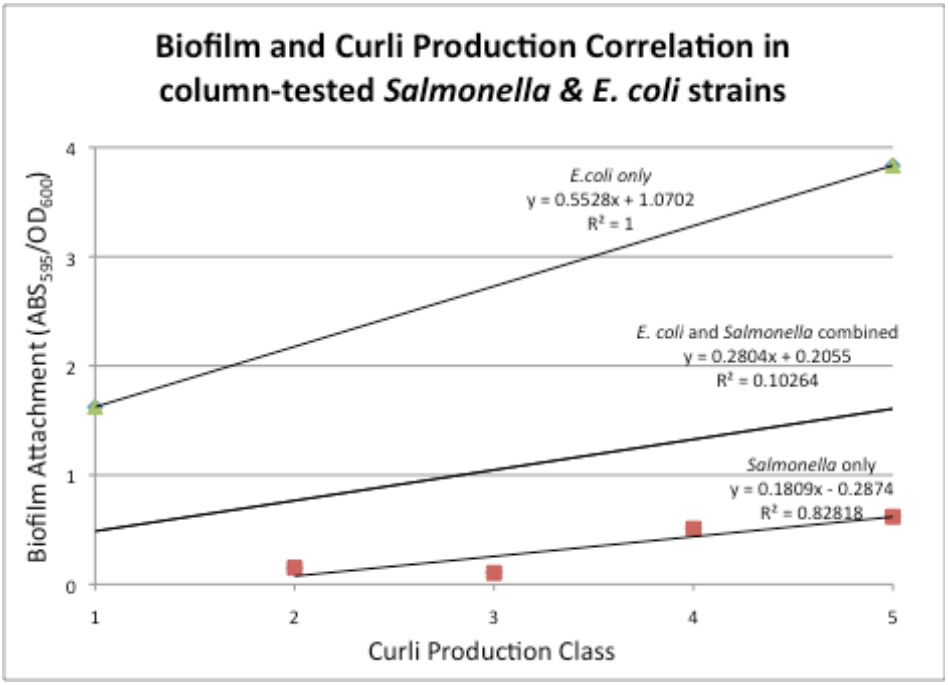


Figure 6: Correlation between biofilm formation and curli production in column-tested subset of *Salmonella* and *E. coli* strains. Linear regression line and equation are shown along with R^2.

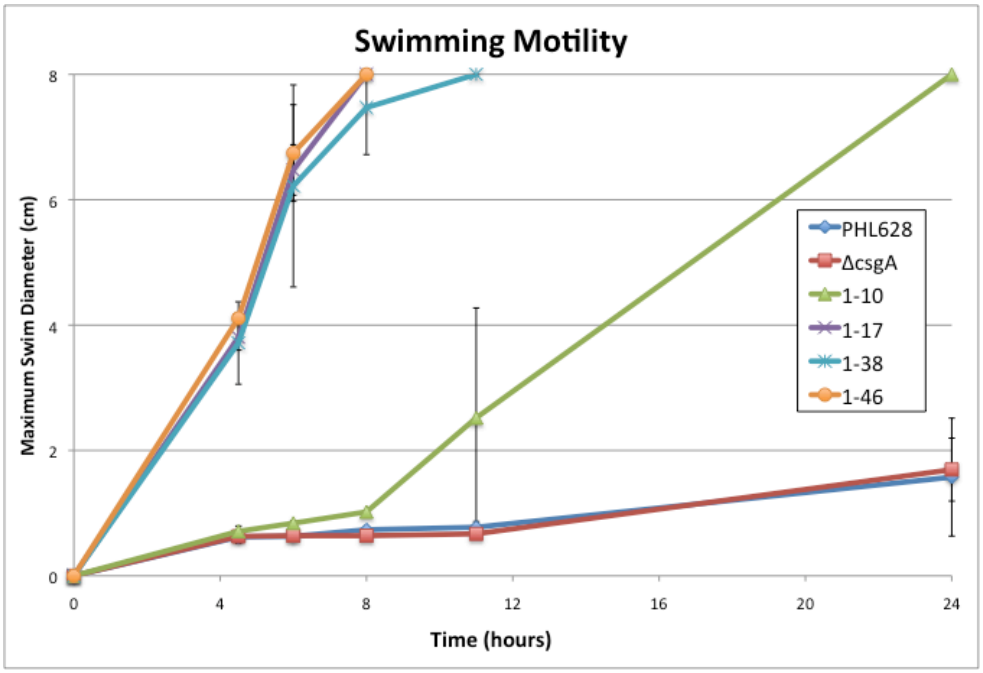
2.3.3 Swimming and Swarming Motility

Swarming and swimming motility are both specialized forms of bacterial surface translocation driven by flagella as the motive force (Henrichsen 1972). In swarming motility, cells move atop the agar surface, whereas in swimming motility, cells move together in a unified front through the agar (Harshay 2003). Figure 6a shows the average swimming motility of four replicates of each strain over time. The tested strains appear to form 3 distinct classes of measured swimming/ ability: fast (1-17, 1-38, 1-46), medium (1-10), and slow (PHL628, *ΔcsgA*) (Figure 7a). The two *E. coli* strains (PHL628 and *ΔcsgA*) were classified as the slow swimming strains, as there was very little motility through the plate by either strain. The *Salmonella* strains were all fast or medium swimmers. The fast swimming strains (1-17, 1-38, 1-46) all moved through the agar quickly and at approximately the same rate. However, strain 1-10 moved slower than the others, at first following the

same pace as the slow swimming *E. coli* strains, before finally becoming more motile. Because it resembles neither the fast nor the slow swimmers, strain 1-10 was placed in its own motility class as a medium swimmer. There was no significant correlation between swimming motility and biofilm formation or curli expression (Appendix C.3).

Unlike the swimming motility, swarming motility was not exhibited by every tested replicate. Even under identical conditions, some replicates of a single strain would swarm across the agar surface, while other replicates would not. This conditional swarming was exhibited by all tested strains. Given that swarming has been shown to be very sensitive to agar conditions (Harshay 2003), we decided to report the swarming abilities of each strain by plotting only the replicates that indeed swarmed (Figure 7b). This figure does not include any of the replicates that showed no swarming. Nonetheless, it does indicate how each strain would swarm when the agar conditions were permissive. The swarming abilities displayed by the strains appear to be linked with swimming, as three classes were again formed by the same strains. *Salmonella sp*. 1-10 again appears to translocate at a slower rate than the other Salmonella strains, while the *E. coli* strains show limited swarming ability. Like the swimming motility, no correlation was observed between swarming motility and biofilm formation or curli production (Appendix C.4).

a.



b.

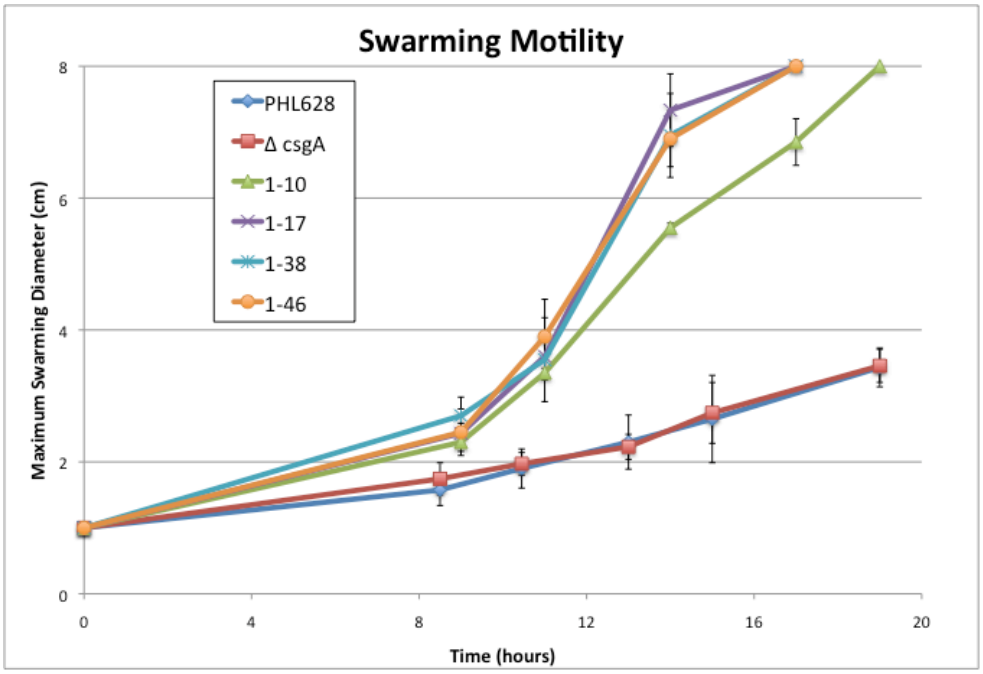


Figure 7: a) Swimming and b) Swarming Motility, measured as maximum diameter swimming across plate over time. Data shown are *E. coli* strains, PHL628 (dark blue diamond) and *ΔcsgA* (red square), and *Salmonella sp. 1-10* (green triangle), *1-17* (purple cross), *1-38* (light blue star), *1-46* (orange circle). Results are a minimum of 3 replicates with error bars representing one standard deviation from the mean.

2.3.4 Sand Column Transport

All strains in the column studies were first grown in LB to increase biomass yield, but which also represses the expression of the curli genes, due to its high osmolarity (Jubelin et al. 2005). To minimize the impact of any biological differences between the strains on transport we resuspended an aliquot in PBS which does not permit growth and therefore does not allow for new extracellular organelles or polysaccharides to be produced. Figure 8 shows the BTCs of all strains in PBS along with that of the conservative tracer (KBr). As expected under these conditions, no significant differences in column effluent concentration were seen between *Salmonella* and *E. coli* strains, despite the differences between strains' ability to form biofilms and produce curli (Figure 8, Appendix B.1). The BTCs of the bacteria showed effluent concentrations with peaks at 40-60% of their initial concentration, yet they maintained a curve similar in shape to that of the KBr tracer BTC. The similarity of shape suggests that the retention of the bacteria in the column was irreversible, at least over the time frame of the experiment (reversible retention would have displayed a lag in the peak tracer concentration (Bales et al. 1997)).

Resuspending an aliquot of cells in M9 growth medium, which is growth permitting, was expected to permit the synthesis of extracellular organelles like curli and allow us to quantify the impact of biological differences between the strains on transport. As expected, the BTCs showed significant differences between the two *E. coli* control strains (Figure 9): PHL628 was significantly more retained ($P < 0.05$) in the column than Δ*csgA* as measured by the mass recovery (Appendix B.2). The Δ*csgA* mutant had much higher effluent measured concentration ratios, with peak bacterial recovery ratios of around 60% as compared to only ~27% for PHL628. The transport differences observed between these two *E. coli* is consistent with

previous findings (Brombacher et al. 2003) and confirm the correlation between biofilms/curli and increased bacterial retention in columns for this *E. coli*.

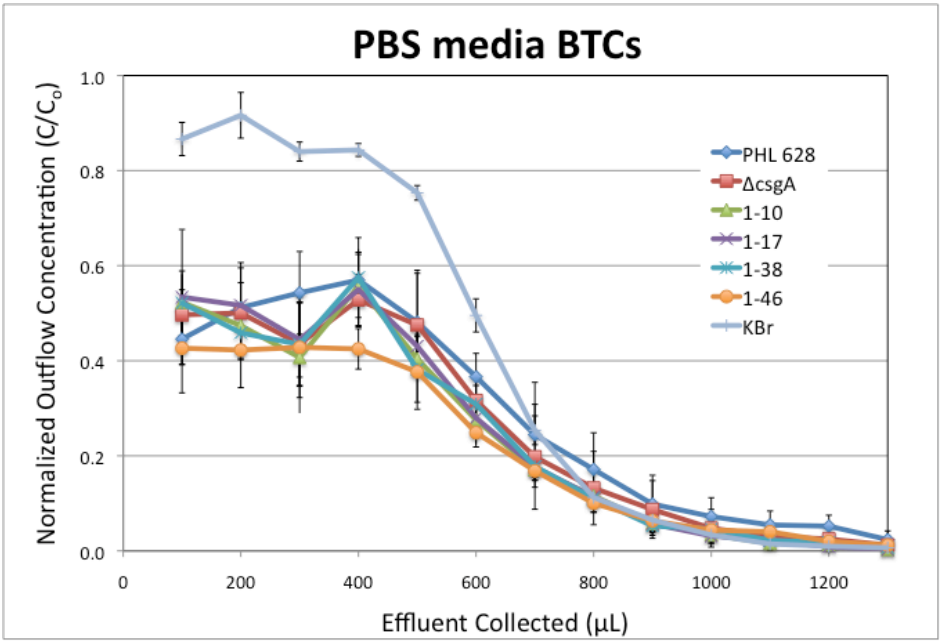


Figure 8: Breakthrough curves of PBS-suspended bacteria sand columns. Data shown are *E. coli* strains, PHL628 (dark blue diamond) and *ΔcsgA* (red square), and *Salmonella sp. 1-10* (green triangle), *1-17* (purple cross), *1-38* (light blue star), *1-46* (orange circle). A conservative tracer, KBr (lavender dash) is also shown. Results are averages from 8 replicate columns, and error bars represent one standard deviation from the mean.

Surprisingly, the BTCs showed the two best biofilm forming *Salmonella* strains (1-38 and 1-46), were poorly retained in the column and had the greatest recovery ratios (of ~76% and ~44% respectively) (Table 3**)**. In fact, *Salmonella* strain 1-38, which congo red plates suggested produced the greatest amounts of curli (as evidenced by its *rdar* morphotype), showed the least retention in the column. In contrast, the strains found to be low biofilm formers (1-10 and 1-17) had percent recoveries statistically similar to 1-46, with the mass recovery of 40% and 43% respectively (Appendix B.2). These *Salmonella* strains which we found to have intermediate congo red morphotypes exhibited similar retention patterns to Δ*csgA* even though they formed about 1/4th as much biofilms as the Δ*csgA* mutant.

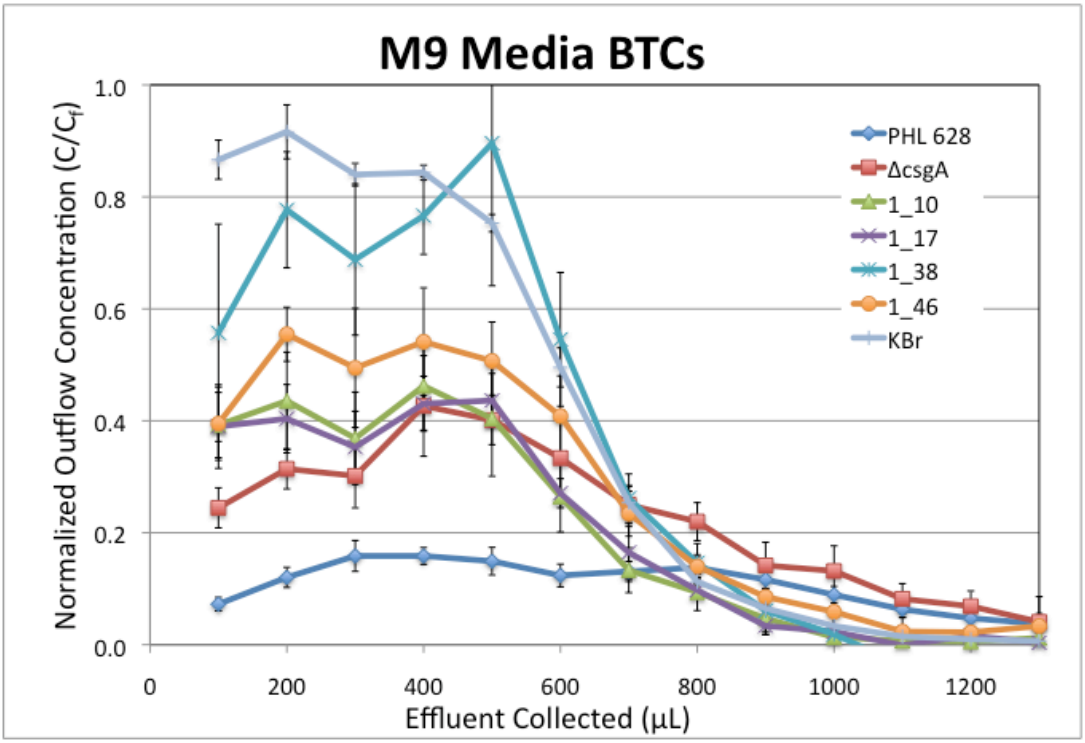


Figure 9: Breakthrough curves of M9-suspended bacteria sand columns. Data shown are *E. coli* strains, PHL628 (dark blue diamond) and *ΔcsgA* (red square), and *Salmonella sp. 1-10* (green triangle), *1-17* (purple cross), *1-38* (light blue star), *1-46* (orange circle). A conservative tracer, KBr (lavender dash) is also shown. Results are averages from 8 replicate columns, and error bars represent one standard deviation from the mean.

Table 2: Summary of all assays

	E. coli			***Salmonella***			
	PHL628	*ΔcsgA*		1-10	1-17	1-38	1-46
Biofilm Attachment (OD_{595}/OD_{600})	3.83	1.62		0.37	0.35	1.69	1.50
Comparative Biofilm Formation	High	Low		Low	Low	High	High
Curli Production	High	None		Low	Low	High	Medium
Cellulose Production	None	None		Low	Low	High	Low
Swimming Motility	None	None		Slow	Fast	Fast	Fast
Swarm Motility	None	None		Slow	Fast	Fast	Fast
M9 Column Mass Recovery	27%	61%		40%	43%	76%	49%
PBS Column Mass Recovery	49%	51%		44%	48%	51%	46%

2.4 *Discussion*

Our initial *Salmonella* collection consisted of 32 strains collected from various dairy farms in the Northeastern United States. As had been previously shown for environmental *E. coli* isolates (Yang et al. 2004), our measurements showed a high amount of diversity with respect to *in vitro* biofilm formation, although we did not find any isolate whose biofilms rivaled those of PHL628 (Figure 3). From this diverse group, four *Salmonella* strains representing the high and low extremes of the isolates' biofilm forming capabilities (Figure 2) were examined in greater detail, with two *E. coli* strains serving as comparative controls for the effect of curli and biofilm formation on transport (Brombacher et al. 2003).

When grown in LB media which is known to repress the production of curli in *E. coli* (Olsén et al. 1993), then resuspended in PBS, neither the *E. coli* nor the *Salmonella* strains showed significant retention differences (Figure 8, Appendix B.1), however, when the cells were resuspended in M9 before being loaded onto the columns we measured significant differences in their retention (Figure 9, Appendix B.2). As expected based on the work of others (Brombacher et al 2003) curli production and enhanced biofilm formation capacity were associated with increased the retention of *E. coli.* Surprisingly, however, curli production and biofilm formation did not correlated with transport of the *Salmonella* strains we analyzed.

Based on the *E. coli* results, we expected the two high biofilm forming *Salmonella* strains (*1-38* & *1-46*) to show higher retention in the columns, as was observed for wild type *E. coli*. However, both of the high biofilm producing strains were *less* strongly retained in the column than either the low biofilm forming strains (*1-10* & *1-17*) or Δ*csgA*, which formed similar biofilms to *1-38* and *1-46*. Although it is tempting to conclude from these results that biofilm formation in *Salmonella* may actually facilitate transport in porous media, the differences in column recovery

rates of the two high biofilm formers (*1-38 & 1-46*), suggests this was not the case. Similarly, production of curli did not hinder transport of *Salmonella* strains, as the highest curli-producing strain (*1-38*) displayed less adherence in the column than the low curli producing strains (*1-10 & 1-17*). In fact, the data suggest that contrary to the results displayed by *E. coli*, production of curli appeared to facilitate transport in *Salmonella.*

As Salmonella spp. *1-38* showed the least amount of retention in the column, and was observed swarming on CR agar (Figure 4e), we hypothesized that a surface translocation mechanism such as swimming or swarming could be overcoming the retentive force of curli and enhancing bacteria transport. Prior studies confirm that bacterial transport in porous media can be facilitated by flagellated motility (McClaine & Ford 2002). However, when all strains were tested specifically for swarming (Figure 7a) and swimming (Figure 7b), our findings show no link between the swimming and swarming ability of a bacterial strain and its transport through sand columns. Therefore, the appearance of swimming or swarming motility does not appear to influence bacterial transport through porous media in our tested strains.

In this work we examined curli production and biofilm formation because they have been shown to be important factors in the transport of *E. coli* through porous media (Brombacher et al. 2003, Stevik et al. 2003). It is possible that the unexpected results we observed are due to the presence of some other differences between our isolates that either override or mask the effect of curli without compromising biofilm formation. For example, lipopolysaccharides, which have been shown to be important for biofilm formation (Genevaux et al. 1999) are also known to facilitate transport (Williams & Fletcher 1996, Bell et al. 2003). Thus overproduction of LPS or some other factor that facilitates biofilm formation may have enhanced transport rather than decreasing it. Our unexpected findings could

also be complicated by the fact that the uptake of congo red dye is not wholly amyloid-specific, since it is also known to bind to cellulose and other exopolysaccharides, rendering the congo red morphotype identification misleading (Khurana et al. 2001). Future work should also include electron microscopy (EM) to complement the congo red morphotype assessment and verify the presence of curli, along with western blotting to quantify curli production.

There are numerous other factors have been shown to impact biofilm formation by environmental *E. coli* (Reisner et al. 2006) and *Salmonella* (Jonas et al. 2007) such as conjugative plasma transfer, cellulose and biofilm associated proteins, whose impact on transport through porous media has not yet been addressed. Additional work is therefore needed to determine if the transport differences we observed are due to LPS or some other factor. Our work also suggests that caution must be applied when extrapolating between genera when it comes to bacterial transport through porous media. Further phenotypic analysis of these strains, and of additional biofilm-forming and/or curli-producing *Salmonella* strains will be required to fully address this question and yield insight into the combination of factors that affect the transport of enterobacteria through unsaturated porous media.

REFERENCES

Abu-Lail N. I. & Camesano T. A. 2003. Role of Lipopolysaccharides in the Adhesion, Retention, and Transport of *Escherichia coli* JM109. Environmental Science & Technology **37:** 2173-2183.

Bales R. C., Li S., Yeh T. C. J., Lenczewski M. E., Gerba C. P. 1997. Bacteriophage and microsphere transport in saturated porous media: Forced-gradient experiment at Borden, Ontario. Water Resources Research. **33:** 639-648.

Barnhart M. M. & Chapman M. R. 2006. Curli Biogenesis and Function. Annual Review of Microbiology **60:** 131-147.

Bell C. H., Bhupinder S. A., Camesano T. A. 2005. Adhesion of *Pseudomonas putida* KT2442 is mediated by surface polymers at the nano- and microscale. Environmental Engineering Science. **22:** 629-641

Bjergbaek L.A. & Roslev P. 2005. Formation of nonculturable *Escherichia coli* in drinking water. Journal of Applied Microbiology **99:** 1090–1098.

Brombacher E., Dorel C., Zehnder A. J., Landini P. 2003. The curli biosynthesis regulator CsgD co-ordinates the expression of both positive and negative determinants for biofilm formation in *Escherichia coli*. Microbiology **149:** 2847–2857.

Burks G. A., Velegol S. B., Paramonova E., Lindenmuth B. E., Feick J. D, Logan B. E. 2003. Macroscopic and nanoscale measurements of the adhesion of bacteria with varying outer layer surface composition. Langmuir **19:** 2366–2371.

Centers for Disease Control and Prevention. 12 Aug. 2008. Investigation of outbreak of infections caused by *Salmonella* Saintpaul. <http://www.cdc.gov/salmonella/saintpaul/>

Colorado Department of Public Health and Environmental. 21 Mar. 2008. New Release "Officials provide update on bottled water order" <http://www.cdphe.state.co.us/release/2008/03212008.html>

Costerton J. W., Lewandowski Z., Caldwell D. E., Korber D. R., Lappin-Scott H. M. 1995. Microbial biofilms. Annual Review of Microbiology **49:** 711–745.

Crane S. R., Westerman P. W., Overcash M. R. 1981. Die-off of fecal indicator organisms following land application of poultry manure. Journal of Environmental Quality **9:** 531-539.

Entry J. A., Hubbard R. K., Theis J. E., Fuhrmann J. J. 2000. The influence of vegetation in riparian filterstrips on coliform bacteria: I. Movement and survival in water. Journal of Environmental Quality **29:** 1206-1214.

Filip Z., Kaddu-Mulindwa D., Milde G. 1988. Survival of some pathogenic and facultative pathogenic bacteria in groundwater. Water Science & Technology **20:** 227-231.

Genevaux P., Bauda P., DuBow M. S., Oudega B. 1999. Identification of Tn10 insertions in the rfaG, rfaP, and galU genes involved lipopolyssacharide core biosynthesis that affect *Escherichia coli* adhesion. Archives of Microbiology **172:** 1-8.

Gerba C. P., Wallis C., Melnick J.L. 1975. Fate of wastewater bacteria and viruses in soil. Journal of Irrigation and Drainage Engineering **101:**157-174.

Ginn T. R., Wood B. D., Nelson K. E., Scheibe T. D., Murphy E. M., Prabhakar Clement T. 2002. Processes in microbial transport in the natural subsurface. Advances in Water Resources **25:** 1017-1042.

Hammar M., Bian Z., Normark S. 1996. Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli*. Proceedings of the National Academy of Sciences of the United States of America **93:** 6562-6566

Harshay R. M., Matsuyama T. 1994. Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: Surface-induced differentiation into hyperflagellate swarmer cells. Proceedings of the National Academy of Sciences of the United States of America. **91:** 8631-8635,

Harshay R. M. 2003. Bacterial motility on a surface: many ways to a common goal. Annual Review of Microbiology **57:** 249-273.

Harvey R. W., Harms H. 2002. Transport of microorganisms in the terrestrial subsurface: in situ and laboratory methods, p. 1138. In: Hurst CJ, (ed.). Manual of environmental microbiology, ASM Press, Washington, DC.

Henrichsen J. 1972. Bacterial surface translocation: a survey and classification. Bacteriological Reviews **36:** 478-503.

Jamieson R. C., Gordon R. J., Sharples K. E., Stratton G. W., Madani A. 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. Canadian Biosystems Engineering **44:** 1.2-1.9

Jonas K., Tomenius H., Kadar A., Normark S., Römling U., Belova L. M., Melefors O. 2007. Roles of curli, cellulose and BapA in *Salmonella* biofilm morphology studied by atomic force microscopy. BMC Microbiology **7:** 70

Jubelin G., Vianney A., Beloin C., Ghigo J. M., Lazzaroni J. C., LeJune P., Dorel C. 2005. CpxR/OmpR interplay regulates curli gene expression in response to osmolarity in *Escherichia coli*. Journal of Bacteriology **187:** 2038–2049.

Kaneene J. B., Warnick L. D., Bolin C. A., Erskine R. J., May K., Miller R. 2008. Changes in tetracycline susceptibility of enteric bacteria following switching to nonmedicated milk replacer for dairy calves. Journal of Clinical Microbiology **46:** 1968-1977

Khurana R., Uversky V. N., Nielsen L., Fink A. L. 2001. Is congo red an amyloid-specific dye? Journal of Biological Chemistry. **25:** 22715-22721.

Landini P., Zehnder A. J. 2002. The global regulatory hns gene negatively affects adhesion to solid surfaces by anaerobically grown *Escherichia coli* by modulating the expression of lipopolysaccharide and flagellar genes. Journal of Bacteriology **184:** 1522-1529.

Macler B. A., Merkle J. C. 2000. Current knowledge on groundwater microbial pathogens and their control. Hydrogeology Journal **8:** 29–40.

McClaine JW, Ford RM. 2002. Characterizing the adhesion of motile and nonmotile *Escherichia coli* to a glass surface using a parallel-plate flow chamber. Biotechnology & Bioengineering **78:** 179–189.

Mezrioui N., Baleux B., Trousselier M. 1995. A microcosm study of the survival of *Escherichia coli* and *Salmonella typhimurium* in brackish water. Water Research **29:** 459–465.

O'Connor R. 2001. Report of the Walkerton Inquiry. Toronto, ON: Publications Ontario. Bioresource Technology **54:**165-169.

Ogden I. D., Fenlon D. R., Vinten A. J. A., Lewis D. 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. International Journal of Food Microbiology. **66:** 111-117.

Olsén A., Arnqvist A., Hammar M., Normark S. 1993. Environmental regulation of curli production in *Escherichia coli*. Infectious Agents and Disease. **2:** 272 – 274.

O'Toole G. A., Kolter R. 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling: a genetic analysis. Molecular Microbiology **29:** 449-461.

Prigent-Combaret C., Brombacher E., Vidal O., Lejeune P., Ambert A., Landini P., Dorel C. 2001. A complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the csgB gene. Journal of Bacteriology **183:** 7213–7223.

Prouty A. M., Schwesinger W. H., Gunn J. S. 2002. Biofilm formation and interaction with the surfaces of gallstone by *Salmonella spp*. Infection & Immunity. **70:** 2640-2649.

Reisner A., Krogfelt K. A., Klein B. M., Zechner K. L., Molin S. 2006. In vitro biofilm formation of commensal and pathogenic *Escherichia coli* strains: impact of environmental and genetic factors. Journal of Bacteriology **188:** 3572–3581.

Rhodes M. W. & Kator H. 1988. Survival of Escherichia coli and Salmonella spp. in estuarine environments. Applied Environmental Microbiology **54:** 2902–2907.

Rijnaarts H. H. M., Norde W., Bouwer E. J., Lyklema J., Zehnder A. J. B. 1995. Reversibility and mechanism of bacterial adhesion. Colloid Surface Biointerfaces **4:** 5–22.

Römling U., Bian Z., Hammar M., Sierralta W., Normark S. 1998. Curli fibers are highly conserved between *Salmonella typhimurium* and *Escherichia coli* with respect to operon structure and regulation. Journal of Bacteriology **180:** 722–731

Sambrook J., Fritsch E. F., Maniatis T. 1989. Molecular Cloning: A Laboratory Manual, Vol. 3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Stevik T. K., Aa K., Ausland G., Hanssen J. F. 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research **38:** 1355–1367.

Toba, F. A. 2008. Characterization of the physiological implications of Defective Lambdoid Phage DLP12 in *Escherichia coli*. PhD Dissertation, Cornell University.

Tufenkji N. 2007. Modeling microbial transport in porous media: Traditional approaches and recent developments. Advances in Water Resources **30:** 1455-1469.

U.S. Environmental Protection Agency. 28 Nov. 2006. Ground Water Rule: Public Health Concerns. < http://www.epa.gov/ogwdw000/standard/phs.html>

Van Houdt R. & Michiels C. 2005. Role of bacterial cell surface structures in *Escherichia coli* biofilm formation. Research in Microbiology **156:** 626–633.

Vidal O., Longin R., Prigent-Combaret C., Dorel C., Hooreman M., Lejeune P. 1998. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression. Journal of Bacteriology **180:** 2442–2491.

Walker S. L., Redman J. A., Elimelech M. 2004. Role of cell surface lipopolysaccharides (LPS) in *Escherichia coli* K12 adhesion and transport. Langmuir **20:** 7736–7746.

Walker S. L., Hill, J. E., Redman, J. A., Elimelech M. 2005. Influence of growth phase on adhesion kinetics of *Escherichia coli* D21g. Applied and Environmental Microbiology **71:** 3093-3099.

White A. P., Gibson D. L., Kim W., Kay W. W., Surette M. G. 2006. Thin aggregative fimbriae and cellulose enhance long-term survival and persistence of *Salmonella*. Journal of Bacteriology **188:** 3219-3227.

Williams V., Fletcher M. 1996. *Pseudomonas fluorescens* adhesion and transport through porous media are affected by lipopolysaccharide composition. Applied Environmental Microbiology **62:** 100-104.

Winfield M. D., Groisman E. A. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. Applied Environmental Microbiology **69:** 3687–3694.

Yang H. H., Vinopal R. T., Grasso D., Smets B. F., 2004. High diversity among environmental Escherichia coli isolates from a bovine feedlot. Applied & Environmental Microbiology **70:** 1528-1536.

Yang H. H., Morrow J. B., Grasso D., Vinopal R. T., Smets B. F. 2006. Intestinal versus external growth conditions changes the surficial properties in a collection of environmental *Escherichia coli* isolates. Environmental Science & Technology **40:** 6976-6982.

Zogaj X., Bokranz W., Nimtz M., Römling U. 2003. Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract. Infection & Immunity **71:** 4151–4158.

CHAPTER 3

RECOMMENDATIONS FOR FUTURE WORK

Based on the results of this initial investigation, we can conclude that the presence of biofilms and curli are not the most important factors influencing transport of environmental *Salmonella*. However, while this conclusion has been supported by our findings, we tested only a small number of *Salmonella* strains. The evaluation of additional Salmonella strains is recommended to verify this finding. In addition, we examined the transport behavior using a novel setup of mini-sand columns. Scaling up these experiments in larger columns would address the question of whether our findings were adversely influenced by the relatively small scale.

Future studies should focus on both the visualization and modeling of bacterial transport processes and the identification of individual transport-influencing factors. Insertion of a fluorescent gene into the bacteria and subsequent observation under a confocal microscope could allow for visualization of transport limiting processes and identify possible attachment sites preferences. Modeling our observed transport would provide useful information about the physical process influencing retention of bacteria. Modeling bacteria like *E. coli* PHL628 and *ΔcsgA* could be useful in developing a retention coefficient to which the presence of biofilms and curli phenotypes could be linked.

A more thorough biological characterization of the strains used in the study could yield value information linking phenotype to trends observed in transport. The observation of cellulose production by *1-38*, poses new questions about the role of cellulose in the transport of *Salmonella*. Further examination of multiple cellulose producing strains in columns is recommended to determine if cellulose has an effect on transport. In addition, the role of LPS still remains unknown. Atomic

force microscopy could be utilized to measure the interactive forces of LPS on the bacterial cell surface. The use of genetically defined LPS mutants in the same *Salmonella* background would also be important since earlier work in *E. coli* was based on strains with different genetic backgrounds (Walker 2004).

Enterobacteria in the environment can show large amounts of genomic and phenotypic heterogeneity (Yang et al. 2004). How this variability relates to the spatial distribution of bacteria in the environment is still unknown. Future experiments examining the bacterial populations found at distinct locations in the hydrologic flowpath for phenotypic characteristics could help determine if specific genotypically controlled surface characteristics influence the transport of bacteria in the environment. In addition, comparing the transport characteristics of bacteria found in the subsurface to bacteria found in the topsoil could yield important information about the hydrologic mechanisms influencing transport, i.e. do bacteria travel through preferential flowpaths.

REFERENCES

Walker S. L., Redman J. A., Elimelech M. 2004. Role of cell surface lipopolysaccharides (LPS) in *Escherichia coli* K12 adhesion and transport. Langmuir **20:** 7736–7746.

Yang H. H., Vinopal R. T., Grasso D., Smets B. F., 2004. High diversity among environmental Escherichia coli isolates from a bovine feedlot. Applied & Environmental Microbiology **70:** 1528-1536.

APPENDIX A

SAND COLUMN EXPERIMENTAL DATA

A.1 July 2, 2008 M9 sand column experimental data

Collected Effluent (µL)	*PHL 628*		*ΔcsgA*		*1-10*	
	Avg C'	St. Dev	Avg C'	St. Dev	Avg C'	St. Dev
100	**0.073**	0.012	**0.244**	0.036	**0.392**	0.058
200	**0.120**	0.018	**0.314**	0.036	**0.436**	0.087
300	**0.159**	0.028	**0.302**	0.057	**0.369**	0.083
400	**0.159**	0.015	**0.426**	0.090	**0.462**	0.079
500	**0.149**	0.025	**0.402**	0.044	**0.404**	0.103
600	**0.124**	0.020	**0.333**	0.072	**0.265**	0.063
700	**0.131**	0.018	**0.250**	0.033	**0.133**	0.040
800	**0.138**	0.019	**0.220**	0.034	**0.094**	0.033
900	**0.116**	0.032	**0.142**	0.041	**0.047**	0.029
1000	**0.089**	0.015	**0.132**	0.045	**0.014**	0.012
1100	**0.063**	0.015	**0.082**	0.027	**0.007**	0.013
1200	**0.047**	0.019	**0.069**	0.027	**0.006**	0.011
1300	**0.037**	0.010	**0.041**	0.015	**0.012**	0.019

Collected Effluent (µL)	*1-17*		*1-38*		*1-46*	
	Avg C'	St. Dev	Avg C'	St. Dev	Avg C'	St. Dev
100	**0.390**	0.075	**0.557**	0.194	**0.395**	0.065
200	**0.404**	0.061	**0.777**	0.104	**0.555**	0.048
300	**0.353**	0.064	**0.688**	0.135	**0.494**	0.106
400	**0.431**	0.049	**0.766**	0.069	**0.541**	0.096
500	**0.436**	0.048	**0.896**	0.255	**0.507**	0.070
600	**0.271**	0.026	**0.546**	0.119	**0.408**	0.072
700	**0.165**	0.046	**0.262**	0.043	**0.234**	0.040
800	**0.098**	0.012	**0.146**	0.034	**0.140**	0.021
900	**0.034**	0.015	**0.061**	0.038	**0.086**	0.026
1000	**0.023**	0.011	**0.019**	0.031	**0.059**	0.026
1100	**-0.001**	0.006	**-0.036**	0.022	**0.024**	0.026
1200	**0.016**	0.033	**-0.050**	0.018	**0.022**	0.028
1300	**0.004**	0.018	**-0.036**	0.086	**0.033**	0.052

A. 2 July 2, 2008 PBS sand column experimental data

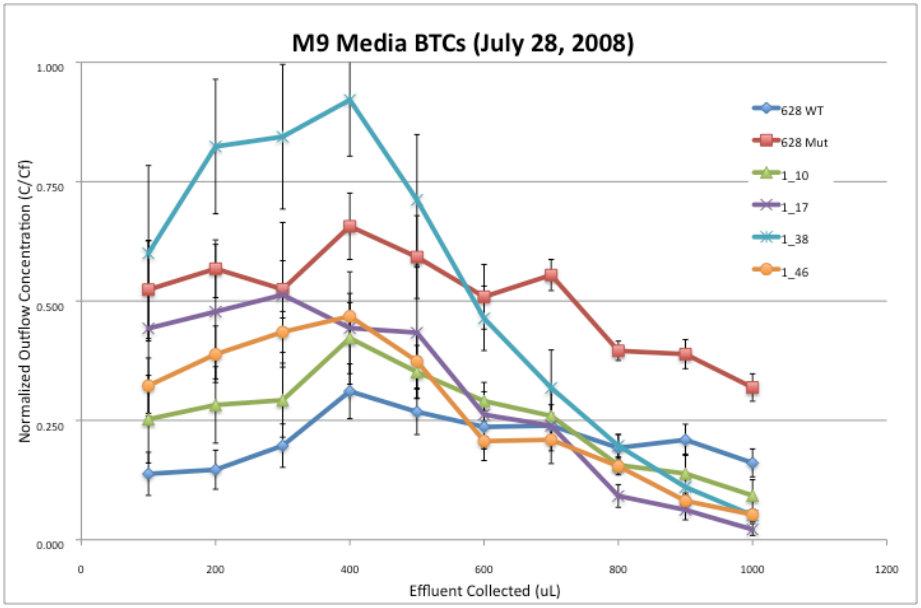
Collected Effluent (µL)	*PHL 628*		*ΔcsgA*		*1-10*	
	Avg C'	St. Dev	Avg C'	St. Dev	Avg C'	St. Dev
100	**0.446**	0.070	**0.497**	0.053	**0.523**	0.060
200	**0.512**	0.045	**0.500**	0.095	**0.474**	0.044
300	**0.543**	0.068	**0.435**	0.087	**0.407**	0.117
400	**0.570**	0.073	**0.528**	0.054	**0.557**	0.078
500	**0.481**	0.069	**0.475**	0.109	**0.404**	0.016
600	**0.366**	0.039	**0.316**	0.049	**0.275**	0.051
700	**0.244**	0.023	**0.198**	0.110	**0.170**	0.023
800	**0.171**	0.028	**0.132**	0.077	**0.116**	0.013
900	**0.098**	0.020	**0.087**	0.061	**0.058**	0.012
1000	**0.072**	0.013	**0.048**	0.040	**0.034**	0.008
1100	**0.055**	0.010	**0.031**	0.029	**0.016**	0.010
1200	**0.052**	0.018	**0.025**	0.023	**0.014**	0.017
1300	**0.024**	0.006	**0.012**	0.018	**0.003**	0.010

Collected Effluent (µL)	*1-17*		*1-38*		*1-46*	
	Avg C'	St. Dev	Avg C'	St. Dev	Avg C'	St. Dev
100	**0.534**	0.142	**0.521**	0.068	**0.426**	0.094
200	**0.516**	0.048	**0.459**	0.056	**0.422**	0.079
300	**0.444**	0.079	**0.434**	0.088	**0.428**	0.106
400	**0.550**	0.078	**0.575**	0.084	**0.425**	0.042
500	**0.431**	0.033	**0.382**	0.069	**0.376**	0.079
600	**0.280**	0.043	**0.309**	0.039	**0.249**	0.030
700	**0.177**	0.028	**0.178**	0.023	**0.168**	0.020
800	**0.114**	0.018	**0.110**	0.028	**0.100**	0.019
900	**0.057**	0.015	**0.053**	0.020	**0.063**	0.009
1000	**0.031**	0.014	**0.046**	0.011	**0.044**	0.016
1100	**0.021**	0.015	**0.022**	0.018	**0.040**	0.017
1200	**0.009**	0.014	**0.018**	0.011	**0.020**	0.011
1300	**0.003**	0.009	**0.007**	0.007	**0.012**	0.004

A. 3 July 2, 2008 M9 sand column experimental data and BTCs

Collected Effluent (µL)	*PHL 628*		*ΔcsgA*		*1-10*	
	Avg C'	St. Dev	Avg C'	St. Dev	Avg C'	St. Dev
100	**0.138**	0.045	**0.524**	0.102	**0.252**	0.092
200	**0.147**	0.041	**0.568**	0.060	**0.282**	0.080
300	**0.197**	0.045	**0.524**	0.060	**0.292**	0.078
400	**0.311**	0.057	**0.657**	0.069	**0.422**	0.074
500	**0.268**	0.048	**0.592**	0.087	**0.351**	0.056
600	**0.236**	0.046	**0.509**	0.068	**0.290**	0.020
700	**0.239**	0.026	**0.554**	0.033	**0.259**	0.023
800	**0.193**	0.028	**0.396**	0.020	**0.156**	0.011
900	**0.209**	0.033	**0.389**	0.030	**0.139**	0.041
1000	**0.161**	0.029	**0.319**	0.029	**0.093**	0.033

Collected Effluent (µL)	*1-17*		*1-38*		*1-46*	
	Avg C'	St. Dev	Avg C'	St. Dev	Avg C'	St. Dev
100	**0.443**	0.109	**0.600**	0.184	**0.323**	0.058
200	**0.478**	0.063	**0.824**	0.141	**0.388**	0.059
300	**0.513**	0.085	**0.844**	0.151	**0.435**	0.043
400	**0.443**	0.094	**0.921**	0.118	**0.468**	0.048
500	**0.434**	0.049	**0.712**	0.137	**0.374**	0.056
600	**0.262**	0.071	**0.464**	0.068	**0.207**	0.041
700	**0.239**	0.021	**0.318**	0.079	**0.209**	0.023
800	**0.092**	0.012	**0.197**	0.024	**0.154**	0.018
900	**0.062**	0.023	**0.110**	0.021	**0.081**	0.012
1000	**0.021**	0.011	**0.051**	0.012	**0.053**	0.010



APPENDIX B

MASS BALANCE DATA

B. 1 Mass Recoveries from PBS-suspended sand columns.

One-Sample Test

	Test Value = 0		
		95% Confidence Interval of the Difference	
	Mean Difference	Lower	Upper
PHL628	.489	.441	.537
Δ*csgA*[a]	.511	.497	.526
1-10	.444	.410	.477
1-17[b]	.475	.463	.486
1-38[b]	.510	.483	.537
1-46[b]	.461	.438	.485

[a] Significantly equal to PHL 628
[b] Salmonella sp. significantly equal to 1-10

B.2 Mass Recoveries from M9-suspended sand columns.

One-Sample Test

	Test Value = 0		
		95% Confidence Interval of the Difference	
	Mean Difference	Lower	Upper
PHL628	.268	.240	.297
Δ*csgA*[a]	.607	.516	.698
1-10[b]	.403	.379	.428
1-17[b]	.431	.414	.447
1-38	.764	.723	.806
1-46[b]	.487	.442	.532

[a] Significantly different from PHL 628
[b] Significantly different from 1-38

APPENDIX C

COMPLETE STATISTICAL DATA

C. 1 PBS Columns Mass Balance Statistics

One-sample student t-tests assuming unequal variances

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
PHL628	16	.48889	.089475	.022369
Delta_csgA	14	.51117	.024841	.006639
Strain_1_10	14	.44355	.057991	.015499
Strain_1_17	14	.47480	.019958	.005334
Strain_1_38	14	.50990	.047356	.012657
Strain_1_46	15	.46150	.042571	.010992

One-Sample T-Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
PHL628	21.856	15	.000	.488885	.44121	.53656
Delta_csgA	76.993	13	.000	.511167	.49682	.52551
Strain_1_10	28.619	13	.000	.443555	.41007	.47704
Strain_1_17	89.014	13	.000	.474802	.46328	.48632
Strain_1_38	40.288	13	.000	.509905	.48256	.53725
Strain_1_46	41.986	14	.000	.461500	.43792	.48507

C. 2 M9 Columns Mass Balance Statistics

One-sample student t-tests assuming unequal variances

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
PHL628	16	.26825	.053191	.013298
Delta_csgA	12	.60687	.143812	.041515
Strain_1_10	15	.40322	.043860	.011325
Strain_1_17	16	.43077	.030802	.007700
Strain_1_38	14	.76412	.072006	.019244
Strain_1_46	14	.48690	.077394	.020684

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
PHL628	20.173	15	.000	.268250	.23991	.29659
Delta_csgA	14.618	11	.000	.606870	.51550	.69824
Strain_1_10	35.605	14	.000	.403216	.37893	.42751
Strain_1_17	55.941	15	.000	.430772	.41436	.44718
Strain_1_38	39.706	13	.000	.764123	.72255	.80570
Strain_1_46	23.540	13	.000	.486902	.44222	.53159

C. 3 Mass Recovery Correlations

Correlation between Column Mass Recoveries and Biological Characteristics for *Salmonella sp.* 1-10, 1-17, 1-38, 1-46 and *E. coli* PHL628 and ΔcsgA. Significance (95% Confidence Interval) ≤ 0.05.

Pearson Correlations

		PBS Mass Balance	M9 Mass Balance	Curli Production	Biofilm Formation	Swim/Swarm Class
PBS Mass Balance	Pearson Correlation	1.000	.426	.042	.309	-.124
	Sig. (2-tailed)		.400	.937	.551	.815
	N	6.000	6	6	6	6
M9 Mass Balance	Pearson Correlation	.426	1.000	-.004	-.600	.621
	Sig. (2-tailed)	.400		.993	.208	.188
	N	6	6.000	6	6	6
Curli Production	Pearson Correlation	.042	-.004	1.000	.320	.332
	Sig. (2-tailed)	.937	.993		.536	.520
	N	6	6	6.000	6	6
Biofilm Formation	Pearson Correlation	.309	-.600	.320	1.000	-.764
	Sig. (2-tailed)	.551	.208	.536		.077
	N	6	6	6	6.000	6
Swim/Swarm Class	Pearson Correlation	-.124	.621	.332	-.764	1.000
	Sig. (2-tailed)	.815	.188	.520	.077	
	N	6	6	6	6	6.000

C. 4 Salmonella biofilm and curli correlations

Pearson Correlation for 32 tested Salmonella strains comparing biofilm formation and curli production

Correlations

		Curli_Production	Biofilm_Formation
Curli_Production	Pearson Correlation	1.000	.750**
	Sig. (2-tailed)		.000
	N	22.000	22
Biofilm_Formation	Pearson Correlation	.750**	1.000
	Sig. (2-tailed)	.000	
	N	22	22.000

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson Correlation for 4 *Salmonella* strains tested in column (1-10, 1-17, 1-38, 1-46)

Correlations

		PBS_Mass_Balance	M9_Mass_Balance	Curli_Produiction	Biofilm_Formation	SwimSwarmClass
PBS_Mass_Balance	Pearson Correlation	1.000	.856	.514	.479	.741
	Sig. (2-tailed)		.144	.486	.521	.259
	N	4.000	4	4	4	4
M9_Mass_Balance	Pearson Correlation	.856	1.000	.617	.766	.497
	Sig. (2-tailed)	.144		.383	.234	.503
	N	4	4.000	4	4	4
Curli_Produiction	Pearson Correlation	.514	.617	1.000	.910	.775
	Sig. (2-tailed)	.486	.383		.090	.225
	N	4	4	4.000	4	4
Biofilm_Formation	Pearson Correlation	.479	.766	.910	1.000	.507
	Sig. (2-tailed)	.521	.234	.090		.493
	N	4	4	4	4.000	4
SwimSwarmClass	Pearson Correlation	.741	.497	.775	.507	1.000
	Sig. (2-tailed)	.259	.503	.225	.493	
	N	4	4	4	4	4.000